Devi, S.
09/151409

-key terms

(FILE 'CAPLUS' ENTERED AT 10:13:07 ON 15 MAY 2001)
L3 33535 SEA FILE=CAPLUS ABB=ON PLU=ON FUSION(S)(PROTEIN OR POLYPROTEIN OR POLYPEPTIDE OR PEPTIDE)
L4 6139 SEA FILE=CAPLUS ABB=ON PLU=ON STREPTOCOCC?(3A)((TYPE OR CLASS OR GROUP)(W)A) OR PEPM OR (STREPTOCOCC? OR PEP)(W)M OR M5 OR SM5 OR M24 OR IMMUNOGEN? PEPTIDE
L15 73 SEA FILE=CAPLUS ABB=ON PLU=ON L3(L)L4
L16 16 SEA FILE=CAPLUS ABB=ON PLU=ON L15(L)RECOMBINAN?

L16 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:814265 CAPLUS
DOCUMENT NUMBER: 133:361906
TITLE: Clostridium botulinum neurotoxin epitopes of all seven serotypes for use in heptavalent vaccine against the toxin
INVENTOR(S): Smith, Leonard A.; Byrne, Michael P.; Middlebrook, John L.; Lapenotiere, Hugh
PATENT ASSIGNEE(S): United States Army Medical Research & Materiel Cmd, USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000067700	A2	20001116	WO 2000-US12890	20000512
WO 2000067700	A3	20010208		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:		
US 1999-133865	P	19990512
US 1999-133866	P	19990512
US 1999-133867	P	19990512
US 1999-133868	P	19990512
US 1999-133869	P	19990512
US 1999-133873	P	19990512
US 1999-146192	P	19990729

AB This invention is directed to prepn. and expression of synthetic genes encoding polypeptides contg. protective epitopes of botulinum

neurotoxin (BoNT). The invention is also directed to prodn. of **immunogenic peptides** encoded by the synthetic genes, as well as recovery and purifn. of the **immunogenic peptides** from **recombinant** organisms. The invention is also directed to methods of vaccination against botulism using the expressed peptides. The epitopes are derived from the C-terminal domain of the heavy chain of the mature toxin and from the central third of the preprotoxins. Manuf. of C-terminal epitopes in Escherichia coli and Pichia pastoris as **fusion** products with maltose-binding **protein** is demonstrated. Extensive modification of codon usage is needed for effective expression in Pichia. Recovery of correctly folded toxin **fusion proteins** (>200-fold purifn., 29% yield) contg. the heavy chain C-terminal epitope of serotype C is demonstrated. The epitope could protect mice against a challenge with 100 LD50's of botulin delivered i.p. Characterization of the immune response and optimization of vaccination protocols is described.

L16 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:348691 CAPLUS
DOCUMENT NUMBER: 133:85434
TITLE: Characterization of the Streptococcal C5a peptidase using a C5a-green fluorescent protein fusion protein substrate
AUTHOR(S): Stafslien, D. K.; Cleary, P. P.
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: J. Bacteriol. (2000), 182(11), 3254-3258
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A glutathione-S-transferase (GST)-C5a-green fluorescent **protein** (GFP) **fusion protein** was designed for use as a substrate for the streptococcal C5a peptidase (SCPA). The substrate was immobilized on a glutathione-Sepharose affinity matrix and used to measure wild-type SCPA activity in the range of 0.8 to 800 nM. The results of the assay demonstrated that SCPA is highly heat stable and has optimal activity on the synthetic substrate at or above pH 8.0. SCPA activity was unaffected by 0.1 to 10 mM Ca2+, Mg2+, and Mn2+ but was inhibited by the same concns. of Zn2+. The assay shows high sensitivity to ionic strength; NaCl inhibits SCPA cleavage of GST-C5a-GFP in a dose-dependent manner. Based on previously published computer homol. modeling, four substitutions were introduced into the putative active site of SCPA: Asp130-Ala, His193-Ala, Asn295-Ala, and Ser512-Ala. All four mutant proteins had over 1,000-fold less proteolytic activity on C5a in

vitro, as detd. both by the GFP assay described here and by a polymorphonuclear cell adherence assay. In addn., **recombinant** SCPA1 and SCPA49, from two distinct lineages of **Streptococcus** pyogenes (**group A streptococci**), and **recombinant** SCPB, from Streptococcus agalactiae (group B streptococci), were compared in the GFP assay. The three enzymes had similar activities, all cleaving approx. 6 mol of C5a mmol of SCP-1 liter-1 min-1.

REFERENCE COUNT: 31

REFERENCE(S):
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:237618 CAPLUS

DOCUMENT NUMBER: 133:57233

TITLE: Improved systems for hydrophobic tagging of recombinant immunogens for efficient iscom incorporation

AUTHOR(S): Andersson, C.; Sandberg, L.; Wernerus, H.; Johansson, M.; Lovgren-Bengtsson, K.; Stahl, S.

CORPORATE SOURCE: Kungliga Tekniska Hogskolan, Department of Biotechnology, Stockholm, S-100 44, Swed.

SOURCE: J. Immunol. Methods (2000), 238(1-2), 181-193
CODEN: JIMMBG; ISSN: 0022-1759

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have previously reported a strategy for prodn. in Escherichia coli of **recombinant** immunogens fused to a hydrophobic tag to improve their capacity to assoc. with an adjuvant formulation [Andersson et al., 1999]. Here, we describe a further development of the previous strategy and present significant improvements. In the novel system, the target immunogen is produced with an N-terminal affinity tag suitable for affinity purifn., and a C-terminal hydrophobic tag, which should enable assocn. through hydrophobic interactions of the immunogen with an adjuvant system, here being immunostimulating complexes (iscoms). Two different hydrophobic tags were evaluated: (i) a tag denoted M, derived from the membrane-spanning region of Staphylococcus aureus protein A (SpA), and (ii) a tag denoted MI consisting of the transmembrane region of hemagglutinin from influenza A virus. Furthermore, two

alternative affinity tags were evaluated; the serum albumin-binding protein ABP, derived from streptococcal protein G, and the divalent IgG-binding ZZ-domains derived from SpA. A malaria peptide **M5**, derived from the central repeat region of the Plasmodium falciparum blood-stage antigen Pf155/RESA, served as model immunogen in this study. Four different **fusion proteins**, ABP-**M5**-M, ABP-**M5**-MI, ZZ-**M5**-M and ZZ-**M5**-MI, were thus produced, affinity purified and evaluated in iscom-incorporation expts. All of the **fusion proteins** were found in the iscom fractions in anal. ultracentrifugation, indicating iscom incorporation. This was further supported by electron microscopy anal. showing that iscoms were formed. In addn., these iscom prepns. were demonstrated to induce **M5**-specific antibody responses upon immunization of mice, confirming the successful incorporation into iscoms. The novel system for hydrophobic tagging of immunogens, with optional affinity and hydrophobic tags, gave expression levels that were increased ten to fifty-fold, as compared to the earlier reported system. We believe that the presented strategy would be a convenient way to achieve efficient adjuvant assocn. for **recombinant** immunogens.

REFERENCE COUNT: 44

REFERENCE(S):

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:560541 CAPLUS

DOCUMENT NUMBER: 131:270662

TITLE: Identification of novel immunogenic Mycobacterium tuberculosis peptides that stimulate mononuclear cells from immune donors

AUTHOR(S): Moran, Alison J.; Doran, James L.; Wu, Jiong; Treit, Janice D.; Ekpo, Pattama; Kerr, Valerie J.; Roberts, Alan D.; Orme, Ian M.; Galant, Shirleen; Ress, Stanley R.; Nano, Francis E.

CORPORATE SOURCE: Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, V8W 3P6, Can.

SOURCE: FEMS Microbiol. Lett. (1999), 177(1), 123-130
CODEN: FMLED7; ISSN: 0378-1097

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteins which are secreted or assocd. with the cell envelope of Mycobacterium tuberculosis may contain protective T-cell epitopes. Prior to this study, a **recombinant** clone bank of enzymically active M. tuberculosis-alk. phosphatase fusions, were screened for immunogenicity in a murine T-cell model. Five of these were selected for further study, and the IFN-.gamma. secretion and proliferation of human PBMC from purified **protein** deriv.-(PPD)-pos. and PPD-neg. donors were measured in response to oligopeptides, Mtb-PhoA **fusions** and one full-length **protein**. Epitopes from four of the five selected antigens were immunoreactive in the human model and corresponded to cytochrome d ubiquinol oxidase, cytochrome c oxidase subunit II, MTV005.02 and MTV033.08. Thus, this strategy identified novel human **immunogenic peptides** as possible candidates for a subunit vaccine.
REFERENCE COUNT: 21
REFERENCE(S):
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 5 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:159061 CAPLUS
DOCUMENT NUMBER: 131:14761
TITLE: Characterization of nra, a global negative regulator gene in group A streptococci
AUTHOR(S): Podbielski, Andreas; Woischnik, Markus; Leonard, Bettina A. B.; Schmidt, Karl-Hermann
CORPORATE SOURCE: Department of Medical Microbiology and Hygiene, University Hospital Ulm, Ulm, D-89081, Germany
SOURCE: Mol. Microbiol. (1999), 31(4), 1051-1064
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB During sequencing of an 11.5 kb genomic region of a serotype M49 **group A streptococcal** (GAS) strain, a series of genes were identified including nra (neg. regulator of GAS). Transcriptional anal. of the region revealed that nra was

primarily monocistronically transcribed. Polycistronic expression was found for the three open reading frames (ORFs) downstream and for the four ORFs upstream of nra. The deduced Nra protein sequence exhibited 62% homol. to the GAS RofA pos. regulator. In contrast to RofA, Nra was found to be a neg. regulator of its own expression and that of the two adjacent operons by anal. of insertional inactivation mutants. By polymerase chain reaction and hybridization assays of 10 different GAS serotypes, the genomic presence of nra, rofA or both was demonstrated. Nra-regulated genes include the fibronectin-binding protein F2 gene (prtF2) and a novel collagen-binding protein (cpa). The Cpa **polypeptide** was purified as a **recombinant** maltose-binding **protein fusion** and shown to bind type I collagen but not fibronectin. In accordance with nra acting as a neg. regulator of prtF2 and cpa, levels of attachment of the nra mutant strain to immobilized collagen and fibronectin was increased above wild-type levels. In addn., nra was also found to regulate neg. (four- to 16-fold) the global pos. regulator gene, mga. Using a strain carrying a chromosomally integrated duplication of the nra 3' end and an nra-luciferase reporter gene transcriptional fusion, nra expression was obsd. to reach its max. during late logarithmic growth phase, while no significant influence of atm. conditions could be distinguished clearly.

REFERENCE COUNT: 53

REFERENCE(S):

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 6 OF 16 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:1970 CAPLUS

DOCUMENT NUMBER: 130:208584

TITLE: Epitope specificities and antibody responses to the EG95 hydatid vaccine

AUTHOR(S): Woollard, D. J.; Gauci, C. G.; Heath, D. D.; Lightowlers, M. W.

CORPORATE SOURCE: Molecular Parasitology Laboratory, The University of Melbourne, Melbourne, 3030, Australia

SOURCE: Parasite Immunol. (1998), 20(11), 535-540 CODEN: PAIMD8; ISSN: 0141-9838

PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Antibody isotype and epitope specificities were examd. in sheep immunized with EG95, a protective **recombinant** vaccine against hydatid disease. All sheep immunized with EG95 as a **fusion protein** with glutathione S-transferase (GST) produced prominent IgG antibodies against the EG95 portion of the **protein**. Linear, antibody-binding epitope specificities of EG95 were mapped using a series of 25 overlapping synthetic peptides. Three immunodominant regions were identified which generated specific IgG1 and IgG2 antibodies in the majority of vaccinated sheep. These regions corresponded to the EG95-derived sequences SLKAVNPSDPLVYKRQTAKF, DIETPRAGKKESTVMTSGSA and SALTSAIAGFVFSC. An addnl. immunogenic region was identified which induced almost exclusively IgG2 antibody. This epitope was located within the sequence TETPLRKHFNLTPV. The anti-parasitic, protective effects of the EG95 vaccine correlated with the detection of specific antibody to two or more of the four linear immunogenic regions. The identification of these **immunogenic peptides** of EG95 maybe useful in the development of a synthetic peptide vaccine as a deriv. of the EG95 **recombinant**.

REFERENCE COUNT: 16
REFERENCE(S):
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(4) Cartwright, G; Journal of Immunological Methods 1995, V179, P31 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:781037 CAPLUS
DOCUMENT NUMBER: 130:236132
TITLE: General expression vectors for production of hydrophobically tagged immunogens for direct iscom incorporation
AUTHOR(S): Andersson, Christin; Sandberg, Lena; Murby, Maria; Sjolander, Anders; Lovgren-Bengtsson, Karin; Stahl, Stefan
CORPORATE SOURCE: Department of Biotechnology, Kungliga Tekniska Hogskolan, Stockholm, S-100 44, Swed.
SOURCE: J. Immunol. Methods (1999), 222(1-2), 171-182

CODEN: JIMMBG; ISSN: 0022-1759
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A new general strategy for the prodn. of **recombinant** protein immunogens has been investigated. The rationale involves the prodn. of a **recombinant** immunogen as fused to a composite tag comprising one domain suitable for affinity purifn. and a hydrophobic tag designed for direct incorporation through hydrophobic interaction of the affinity-purified immunogen into an adjuvant system, in this case immunostimulating complexes (iscoms). Three different hydrophobic tags were evaluated: (i) a tag denoted IW contg. stretches of hydrophobic isoleucine (I) and tryptophan (W) residues; (ii) a tag denoted MI consisting of the transmembrane region of hemagglutinin from influenza A virus; and (iii) a tag denoted PD designed to be pH-dependent in such a way that an amphiphatic .alpha.-helix would be formed at low pH. As an affinity tag, an IgG-binding domain Z derived from Staphylococcus aureus protein A (SpA) was used, and a malaria peptide **M5**, derived from the central repeat region of the Plasmodium falciparum blood-stage antigen Pf155/RESA, served as a model immunogen in this study. Three different **fusion proteins**, IW-Z-**M5**, MI-Z-**M5** and PD-Z-**M5**, were produced in Escherichia coli, and after affinity purifn. these were evaluated in iscom-incorporation expts. Two of the **fusion proteins**, IW-Z-**M5** and MI-Z-**M5** were found in the iscom fraction following preparative ultracentrifugation, indicating iscom incorporation. This was further supported by electron microscopy anal. showing that iscoms were formed. Furthermore, these iscom prepns. were demonstrated to induce efficient **M5**-specific antibody responses upon immunization of mice, confirming successful incorporation into iscoms. The implications of these results for the design and prodn. of subunit vaccines are discussed.

REFERENCE COUNT: 47
REFERENCE(S):
(1) Bradford, M; Anal Biochem 1976, V72, P248 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:983807 CAPLUS

DOCUMENT NUMBER: 124:79818
TITLE: Identification of a plasminogen-binding motif in PAM, a bacterial surface protein
AUTHOR(S): Wistedt, Annika Carlsson; Ringdahl, Ulrika; Mueller-Esterl, Werner; Sjoebring, Ulf
CORPORATE SOURCE: Dep. of Medical Microbiology, Univ. of Lund, Lund, S-223 62, Swed.
SOURCE: Mol. Microbiol. (1995), 18(3), 569-78
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Surface-assocd. plasmin(ogen) may contribute to the invasive properties of various cells. Anal. of plasmin(ogen)-binding surface proteins is therefore of interest. The N-terminal variable regions of M-like (ML) proteins from five different **group A streptococcal** serotypes (33,41,52,53 and 56) exhibiting the plasminogen-binding phenotype were cloned and expressed in Escherichia coli. The **recombinant** proteins all bound plasminogen with high affinity. The binding involved the kringle domains of plasminogen and was blocked by a lysine analog, 6-aminohexanoic acid, indicating that lysine residues in the M-like proteins participate in the interaction. Sequence anal. revealed that the proteins contain common 13-16-amino-acid tandem repeats each with a single central lysine residue. Expts. with **fusion proteins** and a 30-amino-acid synthetic **peptide** demonstrated that these repeats harbor the major plasminogen-binding site in the ML53 **protein**, as well as a binding site for the tissue-type plasminogen activator. Replacement of the lysine in the first repeat with alanine reduced the plasminogen-binding capacity of the ML53 protein by 80%. The results precisely localize the binding domain in a plasminogen surface receptor, thereby providing a unique ligand for the anal. of interactions between kringles and proteins with internal kringle-binding determinants.

L16 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:876271 CAPLUS
DOCUMENT NUMBER: 123:280247
TITLE: Streptokinase-mediated plasminogen activation using a recombinant dual fusion protein construct. A novel approach to study bacterial-host protein interactions
AUTHOR(S): Lizano, Sergio; Johnston, Kenneth H.
CORPORATE SOURCE: Medical Center, Louisiana State University, New Orleans, LA, 70112, USA
SOURCE: J. Microbiol. Methods (1995), 23(3), 261-80
CODEN: JMIMDQ; ISSN: 0167-7012
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Streptokinase (SK), a plasminogen (Pg) activator secreted by **groups A**, C, and G **streptococci**, is extensively used as a pharmacol. agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the mol. basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to study this interaction in which a **recombinant** SK mol. was constructed with glutathione-S-transferase appended to the NH2 terminus and (Gly)3(His)8 appended to the COOH terminus. This dual **fusion protein** mol., immobilized on either Sepharose-S-hexylglutathione or Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK mol. was dependent upon whether the SK mol. was immobilized either at its NH2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the "capture" of intact activator complexes under physiol. conditions and, in addn., may serve as a useful model to analyze other bacterial-host protein interactions important in the pathogenesis of disease.

L16 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:749586 CAPLUS

DOCUMENT NUMBER: 124:46897

TITLE: Characterization of a novel fibronectin-binding surface protein in group A streptococci

AUTHOR(S): Kreikemeyer, B.; Talay, S. R.; Chhatwal, G. S.

CORPORATE SOURCE: Dep. of Microbiology, Technical Univ./GBF-National Research Centre for Biotechnology, Braunschweig, Germany

SOURCE: Mol. Microbiol. (1995), 17(1), 137-45
CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Streptococcus pyogenes interacts with host fibronectin via distinct surface components. One of these components is the Sfbl protein (streptococcal fibronectin-binding protein, now specified as class I), an adhesin that represents a protein family with characteristic features. Here we present the complete structure of a novel fibronectin-binding protein of S. pyogenes, designated SfbII, which is distinct from the previously described Sfbl proteins. The SfbII gene originated from a .lambda. EMBL3 library of chromosomal DNA from **group A streptococcal** strain A75 and coded for a 113 kDa protein exhibiting features of

membrane-anchored surface proteins of Gram-pos. cocci. The expression of biol. active **fusion proteins** allowed the detn. of the location of the fibronectin-binding domain within the C-terminal part of the **protein**. It consisted of two and a half repeats which share common motifs with fibronectin-binding repeats of other streptococcal and staphylococcal proteins. Purified **recombinant fusion protein** contg. this domain competitively inhibited the binding of fibronectin to the parental S. pyogenes strain. Furthermore, polyclonal antibodies against the binding domain specifically blocked the sfbII receptor site on the streptococcal surface. No cross-reactivity could be detected between anti-SfbII antibodies and the sfbI gene product, and vice versa, indicating that the two periods do not share common immunogenic epitopes. Southern hybridization expts. performed with specific sfbII gene probes revealed the presence of the sfbII gene in more than 55% of 93 streptococcal isolates tested. The majority of the strains also harbored the sfbI gene, and 86% carried at least one of the two sfb genes.

L16 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:697873 CAPLUS
DOCUMENT NUMBER: 123:131935
TITLE: In vitro metabolism of terfenadine by a purified recombinant fusion protein containing cytochrome P4503A4 and NADPH-P450 reductase: comparison to human liver microsomes and precision-cut liver tissue slices
AUTHOR(S): Rodrigues, A. D.; Mulford, D. J.; Lee, R. D.; Surber, B. W.; Kukulka, M. J.; Ferrero, J. L.; Thomas, S. B.; Shet, M. S.; Estabrook, R. W.
CORPORATE SOURCE: Abbott Laboratories, Abbott Park, IL, 60064, USA
SOURCE: Drug Metab. Dispos. (1995), 23(7), 765-75
CODEN: DMDSAI; ISSN: 0090-9556
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The metab. of terfenadine was studied with cDNA-expressed/purified **recombinant fusion protein** contg. human liver microsomal cytochrome P 4503A4 (CYP3A4) linked to rat NADPH-P 450 reductase (rF450[mHum3A4/mRatOR]L1) and was compared with that obsd. in the presence of human liver microsomes and precision-cut human liver tissue slices. In all three cases, [3H]terfenadine was metabolized to at least three major metabolites. LC/MS (electrospray) anal. confirmed that these metabolites were .alpha.,.alpha.-diphenyl-4-piperidinomethanol (**M5**), t-Bu hydroxy terfenadine (M4), and t-Bu carboxy terfenadine (M3), although the level of **M5** detected in the presence of **fusion protein** was greater than that found with

microsomes or tissue slices. Two addnl. metabolites, M1 (microsomes and tissue slices) and M2 (**fusion protein**), were also detected, but remain uncharacterized. Consumption of parent drug (microsome: Km = 9.58 .+-. 2.79 .mu.M, Vmax = 801 .+-. 78.3 pmol/min/nmol CYP, **fusion protein**: KM = 14.1 .+-. 1.13 .mu.M, Vmax = 1670 .+-. 170 pmol/min/nmol CYP) and t-Bu hydroxylation to M4 (microsomes: KM = 12.9 .+-. 3.74 .mu.M, Vmax = 643 .+-. 62.5 pmol/min/nmol CYP; **fusion protein**: KM = 30.0 .+-. 2.55 .mu.M, Vmax = 1050 .+-. 141 pmol/min/nmol CYP) obeyed Michaelis-Menten kinetics over the terfenadine concn. range of 1-200 .mu.M. Ketoconazole, a well-documented CYP3A inhibitor, effectively inhibited terfenadine metab. in all three models. The conversion of M4 to M3, studied with human liver microsomes and **fusion protein**, was NADPH-dependent and inhibited by ketoconazole. It is concluded that cDNA-expressed CYP3A4, in the form of a NADPH-P 450 reductase-linked **fusion protein**, may also serve as a model for studying the metab. of terfenadine in vitro and many other drugs.

L16 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:467444 CAPLUS
DOCUMENT NUMBER: 122:211638
TITLE: Oral immunization with the dodecapeptide repeat of the serine-rich Entamoeba histolytica protein (SREHP) fused to the cholera toxin B subunit induces a mucosal and systemic anti-SREHP antibody response
AUTHOR(S): Zhang, Tonghai; Li, Ellen; Stanley, Samuel L., Jr.
CORPORATE SOURCE: Dep. Med., Washington Univ. Sch. Med., St. Louis, MO, 63110, USA
SOURCE: Infect. Immun. (1995), 63(4), 1349-55
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The intestinal protozoan parasite Entamoeba histolytica causes amebic dysentery, a major cause of morbidity worldwide. The induction of a mucosal antibody response capable of blocking amebic adhesion to intestinal cells could represent an approach to preventing E. histolytica infection and disease. Here the authors describe the expression of a chimeric protein contg. an immunogenic dodecapeptide derived from the serine-rich E. histolytica protein (SREHP), fused to the cholera toxin B subunit (CTxB). The CtxB-SREHP-12 chimeric protein was purified from Escherichia coli lysates and retained the crit. GM1 ganglioside-binding activity of the CtxB moiety. Mice fed the CtxB-SREHP-12 **fusion protein** along with a subclin. dose of cholera toxin developed mucosal IgA and IgG and systemic antibody responses that

recognized **recombinant** and native SREHP. The study confirms the feasibility of inducing mucosal immune responses to **immunogenic peptides** by their genetic **fusion** to the CtxB subunit and identifies the CtxB-SREHP-12 chimeric **protein** as a candidate oral vaccine to prevent E. histolytica infection.

L16 ANSWER 13 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:53916 CAPLUS
DOCUMENT NUMBER: 122:152797
TITLE: Cloning, sequencing, and expression of a fibronectin/fibrinogen-binding protein from group A streptococci
AUTHOR(S): Courtney, Harry S.; Li, Yi; Dale, James B.; Hasty, David L.
CORPORATE SOURCE: Veterans Affairs Medical Center, Memphis, TN, 38104, USA
SOURCE: Infect. Immun. (1994), 62(9), 3937-46
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Lipoteichoic acid and several streptococcal proteins have been reported to bind fibronectin (Fn) or fibrinogen (Fgn), which may serve as host receptors. The authors searched for such proteins by screening a library of genes from M type 5 **group A streptococci** cloned into Escherichia coli. Lysates of clones were probed with biotinylated Fn and biotinylated Fgn. One clone expressed a 54-kDa protein that reacted with Fn and Fgn. The protein, termed FBP54, was purified and used to immunize rabbits. Anti-FBP54 serum reacted with purified, **recombinant** FBP54 and with a protein of similar electrophoretic mobility in exts. of M type 5, 6, and 24 streptococci. Anti-FBP54 serum also reacted with 5 of 15 strains of intact, live streptococci, suggesting that FBP54 may be a surface antigen. Southern blot anal. confirmed that the gene is found in **group A streptococci** but not in Staphylococcus aureus or E. coli. The cloned gene was sequenced and contained an open reading frame encoding a protein with a calcd. mol. wt. of 54,186. Partial amino acid sequencing of purified FBP54 confirmed that this open reading frame encoded the protein. As detd. by utilizing **fusion proteins** contg. truncated forms of FBP54, the primary Fn/Fgn-binding domain appears to be contained in residues 1 to 89. These data suggest that FBP54 may be a surface protein of streptococci that reacts with both Fn and Fgn and therefore may participate in the adhesion of **group A streptococci** to host cells.

L16 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:11109 CAPLUS

DOCUMENT NUMBER: 122:26099
TITLE: Localization of immunoglobulin A-binding sites within M or M-like proteins of group A streptococci
AUTHOR(S): Bessen, Debra E.
CORPORATE SOURCE: Sch. Med., Yale Univ., New Haven, CT, 06510, USA
SOURCE: Infect. Immun. (1994), 62(5), 1968-74
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Many strains of **group A streptococci** are capable of binding human IgA (IgA) by a nonimmune mechanism. M or M-like proteins constitute a family of structurally diverse mols. which form surface fibrillae, and some of the M or M-like protein forms are responsible for the IgA-binding activity. In this report, the binding site for IgA is localized within two structurally distinct M or M-like proteins, ML2.2 and Arp4. Apart from those structural domains which are common to all M and M-like proteins, ML2.2 and Arp4 lack significant levels of amino acid sequence homol., with the exception of a short segment (ALXGENXDLR) located at residues 21 to 30 of the mature ML2.2 protein. **Recombinant fusion polypeptides** contg. portions of the ML2.2. and Arp4 **proteins** were expressed in Escherichia coli and tested for binding of human myeloma IgA. A 58-residue polypeptide contg. residues 14 to 71 of ML2.2 bound human IgA. The IgA-binding site of Arp4 could be localized to a 53-residue polypeptide contg. residues 43 to 95, which encompasses the ALXGENXDLR consensus sequence of Arp4 positioned at residues 50 to 59. Site-specific mutagenesis at three codons within the ALXGENXDLR coding sequence of both the ML2.2 and Arp4 **recombinant** polypeptides leads to a loss in IgA-binding activity. Thus, the ALXGENXDLR consensus sequence is essential for the nonimmune binding of IgA by both ML2.2 and Arp4. However, the failure to bind IgA by polypeptides which partially overlap the 58- and 53-residue IgA-binding polypeptides of ML2.2 and Arp4, yet contain the ALXGENXDLR consensus sequence, strongly suggests that flanking regions are also crit. for IgA binding. In summary, the results indicate that common functional domains bearing significant sequence homol. are distributed within regions of M or M-like mols. that are otherwise highly divergent.

L16 ANSWER 15 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:296653 CAPLUS
DOCUMENT NUMBER: 120:296653
TITLE: A method for preparing a kit for the detection of antibodies to HCV (hepatitis C virus) in biological samples such as blood serum
INVENTOR(S): Houghton, Michael; Choo, Qui Lim; Kuo, George

PATENT ASSIGNEE(S): Chiron Corp., India
SOURCE: Indian, 157 pp.
CODEN: INXXAP
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 7
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 171237	A	19920822	IN 1990-CA801	19900917
AU 8927967	A1	19890614	AU 1989-27967	19881118
AU 624105	B2	19920604		
ZA 8808669	A	19890830	ZA 1988-8669	19881118
BR 8807310	A	19900313	BR 1988-7310	19881118
DD 287104	A5	19910214	DD 1988-321971	19881118
IN 169067	A	19910831	IN 1988-CA960	19881118
DD 298524	A5	19920227	DD 1988-344401	19881118
DD 298525	A5	19920227	DD 1988-344402	19881118
DD 298526	A5	19920227	DD 1988-344403	19881118
DD 298527	A5	19920227	DD 1988-344404	19881118
CN 1073719	A	19930630	CN 1992-110257	19881118
JP 05081600	B4	19931115	JP 1989-500565	19881118
JP 09173079	A2	19970708	JP 1996-241451	19881118
JP 09184844	A2	19970715	JP 1996-239921	19881118
JP 10108674	A2	19980428	JP 1997-99651	19881118
JP 10290696	A2	19981104	JP 1998-111631	19881118
JP 10290697	A2	19981104	JP 1998-111632	19881118
JP 2000023683	A2	20000125	JP 1999-157193	19881118
FI 8903447	A	19890717	FI 1989-3447	19890717
NO 8902931	A	19890913	NO 1989-2931	19890717
DK 8903537	A	19890718	DK 1989-3537	19890718
EP 414475	A1	19910227	EP 1990-309120	19900821
EP 414475	B1	19971210		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 161041	E	19971215	AT 1990-309120	19900821
ES 2110411	T3	19980216	ES 1990-309120	19900821
CA 2064705	AA	19910226	CA 1990-2064705	19900822
CA 2064705	C	19990406		
WO 9102820	A1	19910307	WO 1990-US4766	19900822
W: AU, CA, JP				
AU 9063449	A1	19910403	AU 1990-63449	19900822
AU 655156	B2	19941208		
JP 05502156	T2	19930422	JP 1990-512531	19900822
IN 171238	A	19920822	IN 1990-CA802	19900917
IN 171239	A	19920822	IN 1990-CA805	19900917
IN 171240	A	19920822	IN 1990-CA808	19900917
WO 9115771	A1	19911017	WO 1991-US2225	19910329

W: AU, BB, BG, BR, CA, FI, GB, HU, JP, KP, KR, LK, MC, MG, MW, NO, PL, RO, SD, SU
RW: BF, BJ, CF, CG, CM, GA, ML, MR, SN, TD, TG

AU	9176510	A1	19911030	AU 1991-76510	19910329
AU	639560	B2	19930729		
GB	2257784	A1	19930120	GB 1992-20480	19910329
BR	9106309	A	19930420	BR 1991-6309	19910329
HU	62706	A2	19930528	HU 1992-3146	19910329
HU	217025	B	19991129		
JP	05508219	T2	19931118	JP 1991-507636	19910329
JP	2733138	B2	19980330		
RO	109916	B1	19950728	RO 1975-92012	19910329
PL	172133	B1	19970829	PL 1991-296329	19910329
RU	2130969	C1	19990527	RU 1991-5053084	19910329
EP	450931	A1	19911009	EP 1991-302910	19910403
EP	450931	B1	19960612		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE

EP	693687	A1	19960124	EP 1995-114016	19910403
EP	693687	B1	19990728		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE

AT	139343	E	19960615	AT 1991-302910	19910403
ES	2088465	T3	19960816	ES 1991-302910	19910403
AT	182684	E	19990815	AT 1995-114016	19910403
ES	2134388	T3	19991001	ES 1995-114016	19910403
US	5683864	A	19971104	US 1992-910760	19920707
NO	9203839	A	19921119	NO 1992-3839	19921001
US	5714596	A	19980203	US 1993-40564	19930331
LV	10306	B	19950620	LV 1993-442	19930531
LV	10344	B	19960220	LV 1993-4381	19930531
US	5679342	A	19971021	US 1993-97853	19930727
US	5350671	A	19940927	US 1993-103961	19930809
LT	3808	B	19960325	LT 1993-1747	19931230
US	5698390	A	19971216	US 1994-306472	19940915
US	6074816	A	20000613	US 1994-307273	19940916
US	5712087	A	19980127	US 1995-440519	19950512
US	5712088	A	19980127	US 1995-440769	19950515
US	6096541	A	20000801	US 1995-441026	19950515
US	6171782	B1	20010109	US 1995-442647	19950515
US	5863719	A	19990126	US 1995-472821	19950607
NO	9505101	A	19951215	NO 1995-5101	19951215
NO	9505102	A	19951215	NO 1995-5102	19951215
FI	9801380	A	19980615	FI 1998-1380	19980615

PRIORITY APPLN. INFO.:

US	1987-122714	A	19871118
IN	1988-CA960	A	19881118
US	1987-139886	A	19871230
US	1988-161072	A	19880226
US	1988-191263	A	19880506
US	1988-263584	A	19881026

Searcher : Shears 308-4994

US	1988-271450	A	19881114
CN	1988-107988	A	19881118
JP	1992-361785	A3	19881118
JP	1992-361787	A3	19881118
JP	1993-178446	A3	19881118
JP	1996-241451	A3	19881118
JP	1998-111631	A3	19881118
WO	1988-US4125	A	19881118
US	1989-325338	B2	19890317
US	1989-341334	B2	19890420
US	1989-353896	B2	19890421
US	1989-355002	B2	19890518
US	1989-355961	B2	19890518
NO	1989-2931	A	19890717
US	1989-398667	A	19890825
US	1989-456637	B2	19891221
US	1990-504352	A	19900404
US	1990-505435	B2	19900404
US	1990-566209	B1	19900808
WO	1990-US4766	A	19900822
US	1990-611965	B2	19901108
WO	1991-US2225	A	19910329
EP	1991-302910	A3	19910403
US	1992-910760	A3	19920707
US	1993-40564	A3	19930331
US	1993-103961	A1	19930809
US	1994-306472	A3	19940915
US	1994-307273	A3	19940916

AB The title kit contains a (**recombinant**) polypeptide contg. an HCV epitope, a pH buffer, a detection label, assay instructions, and packaging. Also provided are polynucleotide probes for detection of HCV nucleic acids, a monoclonal antibody to an HCV epitope for detection of HCV antigens by immunoassay, and vaccines comprising **immunogenic peptides** contg. an HCV epitope for treatment of HCV infections. The sequence of HCV cDNA suggests that HCV is or resembles a flavivirus. Thus, HCV was isolated from plasma of a chimpanzee with chronic non-A, non-B hepatitis and used to generate a .lambda.-gt11 cDNA library which was screened for prodn. of epitopes which bound to serum from patients with non-A, non-B hepatitis. The cDNAs of several clones were sequenced and used to derive a composite sequence; the corresponding **polypeptides** were expressed in Escherichia coli as **fusion** products with superoxide dismutase.

L16 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:647969 CAPLUS
DOCUMENT NUMBER: 119:247969
TITLE: Hepatitis E virus peptide antigens and

antibodies

INVENTOR(S): Reyes, Gregory R.; Bradley, Daniel W.; Tam, Albert W.; Carl, Mitchell

PATENT ASSIGNEE(S): Genelabs Technologies, Inc., USA; United States Dept. of Health and Human Services

SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 9

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9314116	A1	19930722	WO 1993-US459	19930115
W: CA, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5885768	A	19990323	US 1992-876941	19920501
EP 628053	A1	19941214	EP 1993-903572	19930115
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 08509692	T2	19961015	JP 1993-512710	19930115

PRIORITY APPLN. INFO.:

US 1992-822335	A	19920117
US 1992-876941	A	19920501
US 1988-208997	B2	19880617
US 1989-336672	B2	19890411
US 1989-367486	B2	19890616
US 1989-420921	B2	19891013
US 1990-505888	B2	19900405
US 1991-681078	B2	19910405
WO 1993-US459	W	19930115

AB **Immunogenic peptides** derived from the ORF1, ORF2, and ORF3 regions of hepatitis E virus (HEV) are disclosed for use in diagnostic reagents and vaccines. Antibodies which are immunoreactive with the antigens are also disclosed. Two peptides (406.4-2 from the C-terminus of ORF3; and 406.3-2 from the C-terminus of ORF2) were prepd. by **recombinant** techniques from HEV (Mexico strain) cDNA. The peptides were immunoreactive with human HEV-pos. sera obtained from sources around the world. Vaccines contg. a **fusion protein** of HEV (Burma strain) **peptide** C2 with **recombinant** gene trpE **protein** were prepd. and tested in cynomolgus monkeys.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXLIT, TOXLINE' ENTERED AT 11:37:40 ON 15 MAY 2001)

L20 56 S L16

L21 17 DUP REM L20 (39 DUPLICATES REMOVED)

Searcher : Shears 308-4994

L21 ANSWER 1 OF 17 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2000270157 MEDLINE
DOCUMENT NUMBER: 20270157 PubMed ID: 10809707
TITLE: Characterization of the streptococcal C5a peptidase using a C5a-green fluorescent protein fusion protein substrate.
AUTHOR: Stafslien D K; Cleary P P
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis 55455, USA.
CONTRACT NUMBER: AI20016 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (2000 Jun) 182 (11) 3254-8. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000605

AB A glutathione-S-transferase (GST)-C5a-green fluorescent **protein** (GFP) **fusion protein** was designed for use as a substrate for the streptococcal C5a peptidase (SCPA). The substrate was immobilized on a glutathione-Sepharose affinity matrix and used to measure wild-type SCPA activity in the range of 0.8 to 800 nM. The results of the assay demonstrated that SCPA is highly heat stable and has optimal activity on the synthetic substrate at or above pH 8.0. SCPA activity was unaffected by 0.1 to 10 mM Ca(2+), Mg(2+), and Mn(2+) but was inhibited by the same concentrations of Zn(2+). The assay shows high sensitivity to ionic strength; NaCl inhibits SCPA cleavage of GST-C5a-GFP in a dose-dependent manner. Based on previously published computer homology modeling, four substitutions were introduced into the putative active site of SCPA: Asp(130)-Ala, His(193)-Ala, Asn(295)-Ala, and Ser(512)-Ala. All four mutant **proteins** had over 1,000-fold less proteolytic activity on C5a in vitro, as determined both by the GFP assay described here and by a polymorphonuclear cell adherence assay. In addition, **recombinant** SCPA1 and SCPA49, from two distinct lineages of **Streptococcus** pyogenes (**group A streptococci**), and **recombinant** SCPB, from Streptococcus agalactiae (group B streptococci), were compared in the GFP assay. The three enzymes had similar activities, all cleaving approximately 6 mol of C5a mmol of SCP(-1) liter(-1) min(-1).

L21 ANSWER 2 OF 17 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2000223500 MEDLINE
DOCUMENT NUMBER: 20223500 PubMed ID: 10758248
TITLE: Improved systems for hydrophobic tagging of recombinant immunogens for efficient iscom incorporation.
AUTHOR: Andersson C; Sandberg L; Wernerus H; Johansson M; Lovgren-Bengtsson K; Stahl S
CORPORATE SOURCE: Department of Biotechnology, Kungliga Tekniska Hogskolan, S-100 44, Stockholm, Sweden.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (2000 Apr 21) 238 (1-2) 181-93.
Journal code: IFE; 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000606

AB We have previously reported a strategy for production in Escherichia coli of **recombinant** immunogens fused to a hydrophobic tag to improve their capacity to associate with an adjuvant formulation [Andersson et al., J. Immunol. Methods 222 (1999) 171]. Here, we describe a further development of the previous strategy and present significant improvements. In the novel system, the target immunogen is produced with an N-terminal affinity tag suitable for affinity purification, and a C-terminal hydrophobic tag, which should enable association through hydrophobic interactions of the immunogen with an adjuvant system, here being immunostimulating complexes (iscoms). Two different hydrophobic tags were evaluated: (i) a tag denoted M, derived from the membrane-spanning region of Staphylococcus aureus **protein** A (SpA), and (ii) a tag denoted MI consisting of the transmembrane region of hemagglutinin from influenza A virus. Furthermore, two alternative affinity tags were evaluated; the serum albumin-binding **protein** ABP, derived from streptococcal **protein** G, and the divalent IgG-binding ZZ-domains derived from SpA. A malaria **peptide M5**, derived from the central repeat region of the Plasmodium falciparum blood-stage antigen Pf155/RESA, served as model immunogen in this study. Four different **fusion proteins**, ABP-**M5**-M, ABP-**M5**-MI, ZZ-**M5**-M and ZZ-**M5**-MI, were thus produced, affinity purified and evaluated in iscom-incorporation experiments. All of the **fusion proteins** were found in the iscom fractions in analytical ultracentrifugation, indicating iscom incorporation. This was further supported by electron microscopy analysis showing that iscoms were formed. In addition, these iscom preparations were

demonstrated to induce **M5**-specific antibody responses upon immunisation of mice, confirming the successful incorporation into iscoms. The novel system for hydrophobic tagging of immunogens, with optional affinity and hydrophobic tags, gave expression levels that were increased ten to fifty-fold, as compared to the earlier reported system. We believe that the presented strategy would be a convenient way to achieve efficient adjuvant association for **recombinant** immunogens.

L21 ANSWER 3 OF 17 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999386869 MEDLINE
DOCUMENT NUMBER: 99386869 PubMed ID: 10456923
TITLE: Identification, cloning, and expression of the CAMP factor gene (cfa) of group A streptococci.
AUTHOR: Gase K; Ferretti J J; Primeaux C; McShan W M
CORPORATE SOURCE: Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190, USA.
CONTRACT NUMBER: AI19304 (NIAID)
AI38406 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1999 Sep) 67 (9) 4725-31.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF079502
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991014
Last Updated on STN: 19991014
Entered Medline: 19991005

AB The CAMP reaction is a synergistic lysis of erythrocytes by the interaction of an extracellular **protein** (CAMP factor) produced by some streptococcal species with the Staphylococcus aureus sphingomyelinase C (beta-toxin). **Group A streptococci** (GAS [**Streptococcus** pyogenes]) have been long considered CAMP negative, and this reaction commonly has been used to distinguish GAS from Streptococcus agalactiae. We here provide evidence that GAS possess this gene and produce an extracellular CAMP factor capable of participating in a positive CAMP reaction. The S. pyogenes CAMP factor is specified by a 774-bp open reading frame homologous to the CAMP factor genes from S. agalactiae and Streptococcus uberis. This gene, designated cfa, was isolated on a 1,256-bp fragment and cloned in Escherichia coli. **Recombinant** clones of E. coli expressing cfa secreted an active CAMP factor. The deduced 28.5-kDa **protein** encoded by cfa consists of 257 amino acids, with a predicted 28-amino-acid signal **peptide**. The cfa gene is widely spread among GAS:

82 of 100 clinical GAS isolates produced a positive CAMP reaction. Of the CAMP-negative strains, 17 of the 18 GAS strains contained the cfa gene. Additionally, CAMP activity was detected in streptococci from serogroups C, M, P, R, and U. The cfa gene was cloned and actively expressed in Escherichia coli and gene **fusions** were made, placing the beta-galactosidase gene (lacZ) under control of the cfa promoter. These cfa promoter-lacZ **fusions** were introduced into S. pyogenes via a bacteriophage-derived site-specific integration vector where they showed that the cfa gene has a strong promoter that may be subject to as-yet-unidentified regulatory factors. The results presented here, along with previous reports, indicate that the CAMP factor gene is fairly widespread among streptococci, being present at least in groups A, B, C, G, M, P, R, and U.

L21 ANSWER 4 OF 17 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999195808 MEDLINE
DOCUMENT NUMBER: 99195808 PubMed ID: 10096074
TITLE: Characterization of nra, a global negative regulator gene in group A streptococci.
AUTHOR: Podbielski A; Woischnik M; Leonard B A; Schmidt K H
CORPORATE SOURCE: Department of Medical Microbiology and Hygiene, University Hospital Ulm, Germany.. andreas.podbielski@medizin.uni-ulm.de
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Feb) 31 (4) 1051-64. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990727
Last Updated on STN: 19990727
Entered Medline: 19990715

AB During sequencing of an 11.5 kb genomic region of a serotype M49 **group A streptococcal** (GAS) strain, a series of genes were identified including nra(negative regulator of GAS). Transcriptional analysis of the region revealed that nra was primarily monocistronically transcribed. Polycistronic expression was found for the three open reading frames (ORFs) downstream and for the four ORFs upstream of nra. The deduced Nra **protein** sequence exhibited 62% homology to the GAS RofA positive regulator. In contrast to RofA, Nra was found to be a negative regulator of its own expression and that of the two adjacent operons by analysis of insertional inactivation mutants. By polymerase chain reaction and hybridization assays of 10 different GAS serotypes, the genomic presence of nra, rofA or both was demonstrated. Nra-regulated genes include the fibronectin-binding **protein** F2 gene (prtF2)

and a novel collagen-binding **protein** (cpa). The Cpa **polypeptide** was purified as a **recombinant** maltose-binding **protein fusion** and shown to bind type I collagen but not fibronectin. In accordance with nra acting as a negative regulator of prtF2 and cpa, levels of attachment of the nra mutant strain to immobilized collagen and fibronectin was increased above wild-type levels. In addition, nra was also found to regulate negatively (four- to 16-fold) the global positive regulator gene, mga. Using a strain carrying a chromosomally integrated duplication of the nra 3' end and an nra-luciferase reporter gene transcriptional **fusion**, nra expression was observed to reach its maximum during late logarithmic growth phase, while no significant influence of atmospheric conditions could be distinguished clearly.

L21 ANSWER 5 OF 17 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999144981 MEDLINE
DOCUMENT NUMBER: 99144981 PubMed ID: 10022383
TITLE: General expression vectors for production of hydrophobically tagged immunogens for direct iscom incorporation.
AUTHOR: Andersson C; Sandberg L; Murby M; Sjolander A; Lovgren-Bengtsson K; Stahl S
CORPORATE SOURCE: Department of Biotechnology, Kungliga Tekniska Hogskolan, Stockholm, Sweden.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Jan 1) 222 (1-2) 171-82.
Journal code: IFE; 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990316
Last Updated on STN: 19990316
Entered Medline: 19990302

AB A new general strategy for the production of **recombinant protein** immunogens has been investigated. The rationale involves the production of a **recombinant** immunogen as fused to a composite tag comprising one domain suitable for affinity purification and a hydrophobic tag designed for direct incorporation through hydrophobic interaction of the affinity-purified immunogen into an adjuvant system, in this case immunostimulating complexes (iscoms). Three different hydrophobic tags were evaluated: (i) a tag denoted IW containing stretches of hydrophobic isoleucine (I) and tryptophan (W) residues; (ii) a tag denoted MI consisting of the transmembrane region of hemagglutinin from influenza A virus; and (iii) a tag denoted PD designed to be pH-dependent in such a way

that an amphiphatic alpha-helix would be formed at low pH. As an affinity tag, an IgG-binding domain Z derived from Staphylococcus aureus **protein** A (SpA) was used, and a malaria **peptide M5**, derived from the central repeat region of the Plasmodium falciparum blood-stage antigen Pf155/RESA, served as a model immunogen in this study. Three different **fusion proteins**, IW-Z-**M5**, MI-Z-**M5** and PD-Z-**M5**, were produced in Escherichia coli, and after affinity purification these were evaluated in iscom-incorporation experiments. Two of the **fusion proteins**, IW-Z-**M5** and MI-Z-**M5** were found in the iscom fraction following preparative ultracentrifugation, indicating iscom incorporation. This was further supported by electron microscopy analysis showing that iscoms were formed. Furthermore, these iscom preparations were demonstrated to induce efficient **M5**-specific antibody responses upon immunization of mice, confirming successful incorporation into iscoms. The implications of these results for the design and production of subunit vaccines are discussed.

L21 ANSWER 6 OF 17 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 1999365983 MEDLINE
DOCUMENT NUMBER: 99365983 PubMed ID: 10436930
TITLE: Identification of novel immunogenic Mycobacterium tuberculosis peptides that stimulate mononuclear cells from immune donors.
AUTHOR: Moran A J; Doran J L; Wu J; Treit J D; Ekpo P; Kerr V J; Roberts A D; Orme I M; Galant S; Ress S R; Nano F E
CORPORATE SOURCE: Department of Biochemistry and Microbiology, University of Victoria, Canada.. jmoran@uvic.ca
CONTRACT NUMBER: AI75320 (NIAID)
SOURCE: FEMS MICROBIOLOGY LETTERS, (1999 Aug 1) 177 (1) 123-30.
Journal code: FML; 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990913
Last Updated on STN: 19990913
Entered Medline: 19990902

AB **Proteins** which are secreted or associated with the cell envelope of Mycobacterium tuberculosis may contain protective T-cell epitopes. Prior to this study, a **recombinant** clone bank of enzymatically active M. tuberculosis-alkaline phosphatase **fusions**, were screened for immunogenicity in a murine T-cell

model. Five of these were selected for further study, and the IFN-gamma secretion and proliferation of human PBMC from purified **protein** derivative- (PPD)-positive and PPD-negative donors were measured in response to oligopeptides, Mtb-PhoA **fusions** and one full-length **protein**. Epitopes from four of the five selected antigens were immunoreactive in the human model and corresponded to cytochrome d ubiquinol oxidase, cytochrome c oxidase subunit II, MTV005.02 and MTV033.08. Thus, this strategy identified novel human **immunogenic peptides** as possible candidates for a subunit vaccine.

L21 ANSWER 7 OF 17 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1999140809 MEDLINE
DOCUMENT NUMBER: 99140809 PubMed ID: 9988310
TITLE: Epitope specificities and antibody responses to the EG95 hydatid vaccine.
AUTHOR: Woollard D J; Gauci C G; Heath D D; Lightowlers M W
CORPORATE SOURCE: Molecular Parasitology Laboratory, The University of Melbourne, Werribee, Victoria, Australia.
SOURCE: PARASITE IMMUNOLOGY, (1998 Nov) 20 (11) 535-40. Journal code: OQU; 7910948. ISSN: 0141-9838.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990816
Last Updated on STN: 19990816
Entered Medline: 19990730

AB Antibody isotype and epitope specificities were examined in sheep immunized with EG95, a protective **recombinant** vaccine against hydatid disease. All sheep immunized with EG95 as a **fusion protein** with glutathione S-transferase (GST) produced prominent IgG antibodies against the EG95 portion of the **protein**. Linear, antibody-binding epitope specificities of EG95 were mapped using a series of 25 overlapping synthetic **peptides**. Three immunodominant regions were identified which generated specific IgG1 and IgG2 antibodies in the majority of vaccinated sheep. These regions corresponded to the EG95-derived sequences SLKAVNPSDPLVYKRQTAKF, DIETPRAGKKESTVMTSGSA and SALTSAIAGFVFSC. An additional immunogenic region was identified which induced almost exclusively IgG2 antibody. This epitope was located within the sequence TETPLRKHFNLTPV. The anti-parasitic, protective effects of the EG95 vaccine correlated with the detection of specific antibody to two or more of the four linear immunogenic regions. The identification of these **immunogenic peptides** of EG95 maybe useful in the development of a synthetic **peptide** vaccine as a derivative of the EG95

recombinant.

L21 ANSWER 8 OF 17 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 97101060 MEDLINE
DOCUMENT NUMBER: 97101060 PubMed ID: 8945587
TITLE: Molecular characterization of a major serotype M49 group A streptococcal DNase gene (sdaD).
AUTHOR: Podbielski A; Zarges I; Flosdorff A; Weber-Heynemann J
CORPORATE SOURCE: Institute of Medical Microbiology, Hospital of the Technical University, Aachen, Germany.
SOURCE: INFECTION AND IMMUNITY, (1996 Dec) 64 (12) 5349-56. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X84793; GENBANK-X89235
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19980206
Entered Medline: 19970108

AB **Group A streptococci** (GAS) express up to four types of secreted DNases. Although GAS infections are correlated with the production of anti-DNase B antibodies, the roles of DNases in the pathogenesis of GAS infections remain unclear. From a lambda library of serotype M49 strain CS101 GAS genome, a 2,147-bp fragment expressing DNase activity on an indicator agar was identified and sequenced. One 1,155-bp open reading frame (ORF) was identified in this fragment. This ORF was found to be 48% identical on the amino acid level to group C streptococcal DNase (Sdc). The regions of highest homology corresponded to amino acid residues that were also identified as part of the active site in staphylococcal nuclease. Transcription analysis revealed a specific 1.3-kb mRNA, which corresponded to the size predicted by the promoter and transcription termination signature sequences and indicated a monocistronic mode of transcription. Allelic replacement of the ORF rendered a M49 mutant devoid of extracellular DNase activity when cultured on indicator agar. Virulence parameters such as resistance to phagocytosis were not affected by the mutation. The sda gene was cloned and expressed in Escherichia coli as a thioredoxin **fusion**. By performing Ouchterlony immunodiffusion on the **recombinant protein** and by using **protein** preparations from culture supernatants of wild-type bacteria and the DNase mutant, the results of immunoreactivity with DNase type-specific polyclonal rabbit antisera classified the DNase as a type D enzyme. Fifty percent of patients with sera exhibiting high titers of antistreptolysin or anti-DNase B antibodies also had

SdaD-reactive antibodies in comparison with <10% of serologically normal controls. While the value of **recombinant** SdaD for diagnostic purposes needs to be clarified, the isogenic DNase mutant pair of M49 should allow the significance of GAS DNase D as a bacterial virulence factor to be determined.

L21 ANSWER 9 OF 17 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 95197259 MEDLINE
DOCUMENT NUMBER: 95197259 PubMed ID: 7890393
TITLE: Oral immunization with the dodecapeptide repeat of the serine-rich Entamoeba histolytica protein (SREHP) fused to the cholera toxin B subunit induces a mucosal and systemic anti-SREHP antibody response.
AUTHOR: Zhang T; Li E; Stanley S L Jr
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: AI01231 (NIAID)
DK02072 (NIDDK)
R01AI30084 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1995 Apr) 63 (4) 1349-55.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950427
Last Updated on STN: 19950427
Entered Medline: 19950420

AB The intestinal protozoan parasite Entamoeba histolytica causes amebic dysentery, a major cause of morbidity worldwide. The induction of a mucosal antibody response capable of blocking amebic adhesion to intestinal cells could represent an approach to preventing E. histolytica infection and disease. Here we describe the expression of a chimeric **protein** containing an immunogenic dodecapeptide derived from the serine-rich E. histolytica **protein** (SREHP), fused to the cholera toxin B subunit (CtxB). The CtxB-SREHP-12 chimeric **protein** was purified from Escherichia coli lysates and retained the critical GM1 ganglioside-binding activity of the CtxB moiety. Mice fed the CtxB-SREHP-12 **fusion protein** along with a subclinical dose of cholera toxin developed mucosal immunoglobulin A and immunoglobulin G and systemic antibody responses that recognized **recombinant** and native SREHP. Our study confirms the feasibility of inducing mucosal immune responses to **immunogenic peptides** by their genetic **fusion** to the CtxB subunit and identifies the CtxB-SREHP-12 chimeric **protein** as a candidate oral vaccine to prevent E.

histolytica infection.

L21 ANSWER 10 OF 17 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96089980 MEDLINE
DOCUMENT NUMBER: 96089980 PubMed ID: 7587966
TITLE: In vitro metabolism of terfenadine by a purified recombinant fusion protein containing cytochrome P4503A4 and NADPH-P450 reductase. Comparison to human liver microsomes and precision-cut liver tissue slices.
AUTHOR: Rodrigues A D; Mulford D J; Lee R D; Surber B W; Kukulka M J; Ferrero J L; Thomas S B; Shet M S; Estabrook R W
CORPORATE SOURCE: Department 46V, Abbott Laboratories, Abbott Park, IL 60064, USA.
CONTRACT NUMBER: 16488-25
SOURCE: DRUG METABOLISM AND DISPOSITION, (1995 Jul) 23 (7) 765-75.
Journal code: EBR; 9421550. ISSN: 0090-9556.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19970203
Entered Medline: 19951215

AB The metabolism of terfenadine was studied with a cDNA-expressed/purified **recombinant fusion protein** containing human liver microsomal cytochrome P4503A4 (CYP3A4) linked to rat NADPH-P450 reductase (rF450[mHum3A4/mRatOR]L1) and was compared with that observed in the presence of human liver microsomes and precision-cut human liver tissue slices. In all three cases, [3H]terfenadine was metabolized to at least three major metabolites. LC/MS (electrospray) analysis confirmed that these metabolites were alpha, alpha-diphenyl-4-piperidinomethanol (**M5**), t-butyl hydroxy terfenadine (M4), and t-butyl carboxy terfenadine (M3), although the level of **M5** detected in the presence of **fusion protein** was greater than that found with microsomes or tissue slices. Two additional metabolites, M1 (microsomes and tissue slices) and M2 (**fusion protein**), were also detected, but remain uncharacterized. Consumption of parent drug (microsomes: KM = 9.58 +/- 2.79 microM, Vmax = 801 +/- 78.3 pmol/min/nmol CYP; **fusion protein**: KM = 14.1 +/- 1.13 microM, Vmax = 1670 +/- 170 pmol/min/nmol CYP) and t-butyl hydroxylation to M4 (microsomes: KM = 12.9 +/-3.74 microM, Vmax = 643 +/- 62.5 pmol/min/nmol CYP, ; **fusion protein**

: KM = 30.0 +/- 2.55 microM, Vmax = 1050 +/- 141 pmol/min/nmol CYP) obeyed Michaelis-Menten kinetics over the terfenadine concentration range of 1-200 microM. Ketoconazole, a well-documented CYP3A inhibitor, effectively inhibited terfenadine metabolism in all three models. The conversion of M4 to M3, studied with human liver microsomes and **fusion protein**, was NADPH-dependent and inhibited by ketoconazole. It is concluded that cDNA-expressed CYP3A4, in the form of a NADPH-P450 reductase-linked **fusion protein**, may also serve as a model for studying the metabolism of terfenadine in vitro and many other drugs.

L21 ANSWER 11 OF 17 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 96342385 MEDLINE
DOCUMENT NUMBER: 96342385 PubMed ID: 8748039
TITLE: Identification of a plasminogen-binding motif in PAM, a bacterial surface protein.
AUTHOR: Wistedt A C; Ringdahl U; Muller-Esterl W; Sjobring U
CORPORATE SOURCE: Department of Medical Microbiology, University of Lund, Sweden.
SOURCE: MOLECULAR MICROBIOLOGY, (1995 Nov) 18 (3) 569-78. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19980206
Entered Medline: 19961212

AB Surface-associated plasmin(ogen) may contribute to the invasive properties of various cells. Analysis of plasmin(ogen)-binding surface **proteins** is therefore of interest. The N-terminal variable regions of M-like (ML) **proteins** from five different **group A streptococcal** serotypes (33, 41, 52, 53 and 56) exhibiting the plasminogen-binding phenotype were cloned and expressed in Escherichia coli. The **recombinant proteins** all bound plasminogen with high affinity. The binding involved the kringle domains of plasminogen and was blocked by a lysine analogue, 6-aminohexanoic acid, indicating that lysine residues in the M-like **proteins** participate in the interaction. Sequence analysis revealed that the **proteins** contain common 13-16-amino-acid tandem repeats, each with a single central lysine residue. Experiments with **fusion proteins** and a 30-amino-acid synthetic **peptide** demonstrated that these repeats harbour the major plasminogen-binding site in the ML53 **protein**, as well as a binding site for the tissue-type plasminogen activator. Replacement

of the lysine in the first repeat with alanine reduced the plasminogen-binding capacity of the ML53 **protein** by 80%. The results precisely localize the binding domain in a plasminogen surface receptor, thereby providing a unique ligand for the analysis of interactions between kringles and **proteins** with internal kringle-binding determinants.

L21 ANSWER 12 OF 17 MEDLINE
ACCESSION NUMBER: 96116885 MEDLINE
DOCUMENT NUMBER: 96116885 PubMed ID: 8528052
TITLE: Cytofluorimetric and functional analysis of c-kit receptor in acute leukemia.
AUTHOR: Lauria F; Bagnara G P; Rondelli D; Raspadori D; Strippoli P; Bonsi L; Ventura M A; Montanaro L L; Bubola G; Tura S; +
CORPORATE SOURCE: Istituto di Scienze Mediche, Universita di Milano, Italia.
SOURCE: LEUKEMIA AND LYMPHOMA, (1995 Aug) 18 (5-6) 451-5. Journal code: BNQ; 9007422. ISSN: 1042-8194.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960220
Last Updated on STN: 20000303
Entered Medline: 19960201

AB The SR-1 monoclonal antibody (MoAb) recognizes an epitope of the c-kit receptor (KR), present on normal hemopoietic CD34+ stem cells as well as on blasts from patients with acute leukemia. Cytometric analysis by indirect immunofluorescence with the SR-1 MoAb was performed in 98 patients with acute myeloblastic leukemia (AML) and in 37 patients with acute lymphoblastic leukemia (ALL) in order to detect the presence of the KR and to examine its prognostic significance. Sixty-nine of 98 (70%) AML patients were SR-1 positive independently of the FAB subtype, although a higher incidence of SR-1 positive cases was observed in M4 and **M5** AML and in those cases that also coexpressed lymphoid antigens. Fourteen AML samples were studied by Northern blot analysis and the KR mRNA was detected in the majority of SR-1 positive cases and also in 2 of 3 SR-1 negative samples. Furthermore, "in vitro" cultures from 15 cases showed that **recombinant** human Stem cell factor (rhSCF) induced an increased proliferative activity in most tested cases (11/15); this was further enhanced when rhSCF was combined with rhIL-3 + rhGM-CSF ($p = 0.007$) and with the GM-CSF/IL-3 **fusion protein** PIXY321 ($p = 0.003$). Thirty-seven ALL cases were also studied and all but one were SR-1 negative. Interestingly, the only SR-1 positive case also coexpressed myeloid

antigens and showed an "in vitro" response when stimulated with rhSCF. Finally, the complete remission (CR) rate, survival and event-free survival were evaluated in 75 AML patients who received standard and identical chemotherapy; unlike previous studies which utilized a different anti-KR MoAb (YB5.B8) and which showed a poor prognosis for KR positive patients, we were unable to document any significant difference in CR rate, survival and event-free survival.

L21 ANSWER 13 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 12
ACCESSION NUMBER: 1995:489480 BIOSIS
DOCUMENT NUMBER: PREV199598503780
TITLE: Streptokinase-mediated plasminogen activation using a recombinant dual fusion protein construct. A novel approach to study bacterial-host protein interactions.
AUTHOR(S): Lizano, Sergio; Johnston, Kenneth H. (1)
CORPORATE SOURCE: (1) Dep. Microbiology Immunology Parasitology, La. State Univ. Med. Cent., 1901 Perdido Street, New Orleans, LA 70112 USA
SOURCE: Journal of Microbiological Methods, (1995) Vol. 23, No. 3, pp. 261-280.
ISSN: 0167-7012.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Streptokinase (SK), a plasminogen (Pg) activator secreted by **groups A**, C, and G **streptococci**, is extensively used as a pharmacological agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the molecular basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to study this interaction in which a **recombinant** SK molecule was constructed with glutathione-S-transferase appended to the NH-2 terminus and (Gly)-3(His)-8 appended to the COOH terminus. This dual **fusion protein** molecule, immobilized on either Sepharose-S-hexylglutathione or Ni-2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK molecule was dependent upon whether the SK molecule was immobilized either at its NH-2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the 'capture' of intact activator complexes under physiological conditions and, in addition, may serve as a useful model to analyze other bacterial-host **protein** interactions important in the pathogenesis of disease.

L21 ANSWER 14 OF 17 MEDLINE DUPLICATE 13

ACCESSION NUMBER: 96020668 MEDLINE
DOCUMENT NUMBER: 96020668 PubMed ID: 7476200
TITLE: Characterization of a novel fibronectin-binding surface protein in group A streptococci.
AUTHOR: Kreikemeyer B; Talay S R; Chhatwal G S
CORPORATE SOURCE: Department of Microbiology, Technical University/GBF-National Research Centre for Biotechnology, Braunschweig, Germany.
SOURCE: MOLECULAR MICROBIOLOGY, (1995 Jul) 17 (1) 137-45.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X83303
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951218

AB Streptococcus pyogenes interacts with host fibronectin via distinct surface components. One of these components is the Sfbl **protein** (streptococcal fibronectin-binding **protein**, now specified as class I), an adhesin that represents a **protein** family with characteristic features. Here we present the complete structure of a novel fibronectin-binding **protein** of S. pyogenes, designated Sfbll, which is distinct from the previously described Sfbl **proteins**. The sfbll gene originated from a lambda EMBL3 library of chromosomal DNA from **group A streptococcal** strain A75 and coded for a 113 kDa **protein** exhibiting features of membrane-anchored surface **proteins** of Gram-positive cocci. The expression of biologically active **fusion proteins** allowed the determination of the location of the fibronectin-binding domain within the C-terminal part of the **protein**. It consisted of two and a half repeats which share common motifs with fibronectin-binding repeats of other streptococcal and staphylococcal **proteins**. Purified **recombinant fusion protein** containing this domain competitively inhibited the binding of fibronectin to the parental S. pyogenes strain. Furthermore, polyclonal antibodies against the binding domain specifically blocked the Sfbll receptor site on the streptococcal surface. No cross-reactivity could be detected between anti-Sfbll antibodies and the sfbl gene product, and vice versa, indicating that the two **proteins** do not share common immunogenic epitopes. Southern hybridization experiments performed with specific sfbll gene probes revealed the presence of the sfbll gene in more than 55% of 93 streptococcal

isolates tested. The majority of the strains also harboured the sfb1 gene, and 86% carried at least one of the two sfb genes.

L21 ANSWER 15 OF 17 MEDLINE DUPLICATE 14

ACCESSION NUMBER: 94341902 MEDLINE
DOCUMENT NUMBER: 94341902 PubMed ID: 8063411
TITLE: Cloning, sequencing, and expression of a fibronectin/fibrinogen-binding protein from group A streptococci.
AUTHOR: Courtney H S; Li Y; Dale J B; Hasty D L
CORPORATE SOURCE: Veterans Affairs Medical Center, Memphis, Tennessee 38104.
CONTRACT NUMBER: AI-10085 (NIAID)
DE-07218 (NIDCR)
SOURCE: INFECTION AND IMMUNITY, (1994 Sep) 62 (9) 3937-46.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L28919
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 20000303
Entered Medline: 19940921

AB Lipoteichoic acid and several streptococcal **proteins** have been reported to bind fibronectin (Fn) or fibrinogen (Fgn), which may serve as host receptors. We searched for such **proteins** by screening a library of genes from M type 5 **group A streptococci** cloned into Escherichia coli. Lysates of clones were probed with biotinylated Fn and biotinylated Fgn. One clone expressed a 54-kDa **protein** that reacted with Fn and Fgn. The **protein**, termed FBP54, was purified and used to immunize rabbits. Anti-FBP54 serum reacted with purified, **recombinant** FBP54 and with a **protein** of similar electrophoretic mobility in extracts of M type 5, 6, and 24 streptococci. Anti-FBP54 serum also reacted with 5 of 15 strains of intact, live streptococci, suggesting that FBP54 may be a surface antigen. Southern blot analysis confirmed that the gene is found in **group A streptococci** but not in Staphylococcus aureus or E. coli. The cloned gene was sequenced and contained an open reading frame encoding a **protein** with a calculated molecular weight of 54,186. Partial amino acid sequencing of purified FBP54 confirmed that this open reading frame encoded the **protein**. As determined by utilizing **fusion proteins** containing truncated forms of FBP54, the primary Fn/Fgn-binding domain appears to be contained in residues 1 to 89. These data suggest that FBP54 may be a surface **protein** of

streptococci that reacts with both Fn and Fgn and therefore may participate in the adhesion of **group A streptococci** to host cells.

L21 ANSWER 16 OF 17 MEDLINE DUPLICATE 15

ACCESSION NUMBER: 94222570 MEDLINE
DOCUMENT NUMBER: 94222570 PubMed ID: 8168964
TITLE: Localization of immunoglobulin A-binding sites within M or M-like proteins of group A streptococci.
AUTHOR: Bessen D E
CORPORATE SOURCE: Department of Epidemiology and Public Health (Microbiology Section), Yale University School of Medicine, New Haven, Connecticut 06510.
CONTRACT NUMBER: AI-28944 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1994 May) 62 (5) 1968-74. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940613
Last Updated on STN: 19940613
Entered Medline: 19940602

AB Many strains of **group A streptococci** are capable of binding human immunoglobulin A (IgA) by a nonimmune mechanism. M or M-like **proteins** constitute a family of structurally diverse molecules which form surface fibrillae, and some of the M or M-like **protein** forms are responsible for the IgA-binding activity. In this report, the binding site for IgA is localized within two structurally distinct M or M-like **proteins**, ML2.2 and Arp4. Apart from those structural domains which are common to all M and M-like **proteins**, ML2.2 and Arp4 lack significant levels of amino acid sequence homology, with the exception of a short segment (ALXGENXDLR) located at residues 21 to 30 of the mature ML2.2 **protein**. **Recombinant fusion polypeptides** containing portions of the ML2.2 and Arp4 **proteins** were expressed in Escherichia coli and tested for binding of human myeloma IgA. A 58-residue **polypeptide** containing residues 14 to 71 of ML2.2 bound human IgA. The IgA-binding site of Arp4 could be localized to a 53-residue **polypeptide** containing residues 43 to 95, which encompasses the ALXGENXDLR consensus sequence of Arp4 positioned at residues 50 to 59. Site-specific mutagenesis at three codons within the ALXGENXDLR coding sequence of both the ML2.2 and Arp4 **recombinant polypeptides** leads to a loss in IgA-binding activity. Thus, the ALXGENXDLR consensus sequence is essential for the nonimmune binding of IgA by

both ML2.2 and Arp4. However, the failure to bind IgA by **polypeptides** which partially overlap the 58- and 53-residue IgA-binding **polypeptides** of ML2.2 and Arp4, yet contain the ALXGENXDLR consensus sequence, strongly suggests that flanking regions are also critical for IgA binding. In summary, the results indicate that common functional domains bearing significant sequence homology are distributed within regions of M or M-like molecules that are otherwise highly divergent.

L21 ANSWER 17 OF 17 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 89271871 MEDLINE
DOCUMENT NUMBER: 89271871 PubMed ID: 2499253
TITLE: Complete secretion of activable bovine prochymosin by genetically engineered L forms of Proteus mirabilis.
AUTHOR: Klessen C; Schmidt K H; Gumpert J; Grosse H H; Malke H
CORPORATE SOURCE: Central Institute of Microbiology and Experimental Therapy, Academy of Sciences of the German Democratic Republic, Jena.
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1989 Apr) 55 (4) 1009-15.
Journal code: 6K6; 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198907
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19890711

AB To circumvent problems encountered in the synthesis of active chymosin in a number of bacteria and fungi, a **recombinant** DNA L-form expression system that directed the complete secretion of fully activable prochymosin into the extracellular culture medium was developed. The expression plasmid constructions involved the in-frame **fusion** of prochymosin cDNA minus codons 1 to 4 to **streptococcal** pyrogenic exotoxin **type A** gene (speA') sequences, including the speA promoter, ribosomal binding site, and signal sequence and five codons of mature SpeA. Secretion of **fusion** prochymosin enzymatically and immunologically indistinguishable from bovine prochymosin was achieved after transformation of two stable protoplast type L-form strains derived from Proteus mirabilis. The secreted proenzyme was converted by autocatalytic processing to chymosin showing milk-clotting activity. In controlled laboratory fermentation processes, a maximum specific rate of activable prochymosin synthesis of 0.57 x 10(-3)/h was determined from the time courses of biomass dry weight and product formation. Yields as high as 40 +/-

10 micrograms/ml were obtained in the cell-free culture fluid of strain L99 carrying a naturally altered expression plasmid of increased segregational stability. The expression-secretion system described may be generally useful for production of **recombinant** mammalian **proteins** synthesized intracellularly as aberrantly folded insoluble aggregates.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, JICST-EPLUS, PHIC, PHIN, TOXLIT, TOXLINE' ENTERED AT 12:03:14 ON 15 MAY 2001)

L22 2488 S DALE J?/AU - Author

L24 14 S L22 AND L15
L25 7 DUP REM L24 (7 DUPLICATES REMOVED)

L25 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:316649 CAPLUS
DOCUMENT NUMBER: 132:333387
TITLE: Recombinant multivalent M protein vaccine against Streptococcus
INVENTOR(S): **Dale, James B.**; Lederer, James W.
PATENT ASSIGNEE(S): University of Tennessee Research Corporation, USA
SOURCE: U.S., 62 pp., Cont.-in-part of U.S. Ser. No. 945,954, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6063386	A	20000516	US 1997-937271	19970915
PRIORITY APPLN. INFO.:			US 1992-945954 B2	19920916

AB The authors disclose the prepn. of chimeric matrix proteins derived from multiple serotypes of group A streptococci. The chimeric proteins are immunogenic and provoke opsonic antibodies in rabbits.
REFERENCE COUNT: 85
REFERENCE(S):
(3) Baird; The Journal Of Immunology 1991, V146(9), P3132 CAPLUS
(4) Beachey; US 4284537 1981 CAPLUS
(5) Beachey; US 4454121 1984 CAPLUS
(6) Beachey; US 4521334 1985 CAPLUS
(7) Beachey; US 4597967 1986 CAPLUS
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L25 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:194270 CAPLUS

DOCUMENT NUMBER: 130:236454
TITLE: Streptococcus Group A vaccines containing hexavalent protein M
INVENTOR(S): **Dale, James B.**
PATENT ASSIGNEE(S): ID Vaccine, USA
SOURCE: PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9913084	A1	19990318	WO 1998-US19100	19980914
W: AL, AM, AT, AU, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9893884	A1	19990329	AU 1998-93884	19980914
EP 1003875	A1	20000531	EP 1998-946991	19980914
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1997-58635 P 19970912
WO 1998-US19100 W 19980914

AB The invention provides synthetic **fusion polypeptides**, comprising two or more immunogenic **polypeptides** in length at least 10 amino acids from **Streptococcus Group A**, stimulate an immune response against **streptococcus Group A** while the C terminus of **polypeptides** are not required for its immunogenicity. The mouse protection assay demonstrated that the hexavalent protein M vaccines are effective.

REFERENCE COUNT: 6
REFERENCE(S):
(1) Beachey, E; JOURNAL OF EXPERIMENTAL MEDICINE 1986, V163(6), P1451 CAPLUS
(2) Beachey, E; JOURNAL OF EXPERIMENTAL MEDICINE 1987, V166(3), P647 CAPLUS
(3) Dale, J; JOURNAL OF IMMUNOLOGY 1993, V151(4), P2188 CAPLUS
(4) Dale, J; VACCINE 1996, V14(10), P944 CAPLUS
(5) Univ Tennessee Res Corp; WO 9406421 A 1994 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

Searcher : Shears 308-4994

L25 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 1995:543762 CAPLUS
DOCUMENT NUMBER: 122:312508
TITLE: Intranasal immunization with recombinant group A streptococcal M protein fragment fused to the B subunit of Escherichia coli labile toxin protects mice against systemic challenge infections
AUTHOR(S): **Dale, James B.**; Chiang, Elbert C.
CORPORATE SOURCE: Department of Veterans Affairs Medical Center, Memphis, TN, 38104, USA
SOURCE: J. Infect. Dis. (1995), 171(4), 1038-41
CODEN: JIDIAQ; ISSN: 0022-1899
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A **fusion** gene named LT-B-**M5** was constructed encoding the entire B subunit of Escherichia coli labile toxin (LT-B), a 7 amino acid proline-rich linker, and 15 N-terminal amino acids of type 5 **streptococcal M protein** . The purified LT-B-**M5** was immunogenic in rabbits and evoked antibodies against a synthetic peptide copy of the N-terminus of **M5** (**SM5**[1-15]) and the native **M5** protein and opsonic antibodies against type 5 streptococci. The hybrid protein retained the ganglioside-binding activity of LT-B and was tested in mice for its immunogenicity after local administration. Mice that were immunized intranasally with LT-B-**M5** developed serum antibodies against **SM5**(1-15) and were significantly protected from death after i.p. challenge infections with type 5 streptococci. The data show that protective systemic immune responses may be evoked after intranasal immunization with a fragment of M protein fused to LT-B.

L25 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:465547 CAPLUS
DOCUMENT NUMBER: 121:65547
TITLE: Antigen of hybrid m protein and carrier for group a streptococcal vaccine
INVENTOR(S): **Dale, James B.**
PATENT ASSIGNEE(S): Univesity of Tennessee Research Corp., USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9406465	A1	19940331	WO 1993-US8704	19930915
W: AU, CA, CZ, FI, HU, JP, KR, NO, NZ, PL, RU, SK				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 618813	A1	19941012	EP 1993-922202	19930915
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
PRIORITY APPLN. INFO.:			US 1992-945860	19920916
			WO 1993-US8704	19930915

AB **Streptococcal M protein** **peptides** that elicit protective antibodies against **Group A streptococci** and prevent rheumatic fever are manufd. as **fusion proteins** of N- and C-terminal **peptides** of the **protein** by expression of the gene in a microbial host. The peptides used may be shorter than those normally required for vaccines. Peptides from other proteins may be used as the carrier with the domains linked by a hydrophobic peptide. The protein may be administered by conventional methods, or by use of a non-pathogenic Streptococcus, e.g. a non-cariogenic S. mutans, expressing the gene. **Fusion** products of the **M24 protein** and the B subunit of Escherichia coli heat-labile enterotoxin were manufd. by expression of the gene in Escherichia coli. The proteins were purified, emulsified with complete Freund's adjuvant and 300 .mu.g of protein injected s.c. into rabbits with a booster given four weeks later. Specific opsonic antibodies against type 24 Streptococcus were obtained; these antibodies were not effective against type 5 Streptococcus. In passive mouse protection tests, the i.p. LD50 for type 24 Streptococcus was 1.5.times.105 CFU for control animals and 2.5.times.106 for animals pretreated with rabbit antiserum.

L25 ANSWER 5 OF 7 TOXLIT
ACCESSION NUMBER: 1994:91807 TOXLIT
DOCUMENT NUMBER: CA-121-065547V
TITLE: Antigen of hybrid m protein and carrier for group a streptococcal vaccine.
AUTHOR: **Dale JB**
SOURCE: (1994). PCT Int. Appl. PATENT NO. 94 06465 03/31/94 (Univesity of Tennessee Research Corp.).
PUB. COUNTRY: United States
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 121:65547
ENTRY MONTH: 199409
AB **Streptococcal M protein**

peptides that elicit protective antibodies against **Group A streptococci** and prevent rheumatic fever are manufd. as **fusion proteins** of N- and C-terminal **peptides** of the **protein** by expression of the gene in a microbial host. The **peptides** used may be shorter than those normally required for vaccines. **Peptides** from other **proteins** may be used as the carrier with the domains linked by a hydrophobic **peptide**. The **protein** may be administered by conventional methods, or by use of a non-pathogenic Streptococcus, e.g. a non-cariogenic S. mutans, expressing the gene. **Fusion** products of the **M24 protein** and the B subunit of Escherichia coli heat-labile enterotoxin were manufd. by expression of the gene in Escherichia coli. The **proteins** were purified, emulsified with complete Freund's adjuvant and 300 mug of **protein** injected s.c. into rabbits with a booster given four weeks later. Specific opsonic antibodies against type 24 Streptococcus were obtained; these antibodies were not effective against type 5 Streptococcus. In passive mouse protection tests, the i.p. LD50 for type 24 Streptococcus was 1.5.times.105 CFU for control animals and 2.5.times.106 for animals pretreated with rabbit antiserum.

L25 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2

ACCESSION NUMBER: 1995:53916 CAPLUS
DOCUMENT NUMBER: 122:152797
TITLE: Cloning, sequencing, and expression of a fibronectin/fibrinogen-binding protein from group A streptococci
AUTHOR(S): Courtney, Harry S.; Li, Yi; **Dale, James B.**; Hasty, David L.
CORPORATE SOURCE: Veterans Affairs Medical Center, Memphis, TN, 38104, USA
SOURCE: Infect. Immun. (1994), 62(9), 3937-46
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Lipoteichoic acid and several streptococcal proteins have been reported to bind fibronectin (Fn) or fibrinogen (Fgn), which may serve as host receptors. The authors searched for such proteins by screening a library of genes from M type 5 **group A streptococci** cloned into Escherichia coli. Lysates of clones were probed with biotinylated Fn and biotinylated Fgn. One clone expressed a 54-kDa protein that reacted with Fn and Fgn. The protein, termed FBP54, was purified and used to immunize rabbits. Anti-FBP54 serum reacted with purified, recombinant FBP54 and with a protein of similar electrophoretic mobility in exts. of M type 5, 6, and 24 streptococci. Anti-FBP54 serum also reacted with 5 of 15 strains of intact, live streptococci, suggesting that FBP54 may be a

surface antigen. Southern blot anal. confirmed that the gene is found in **group A streptococci** but not in Staphylococcus aureus or E. coli. The cloned gene was sequenced and contained an open reading frame encoding a protein with a calcd. mol. wt. of 54,186. Partial amino acid sequencing of purified FBP54 confirmed that this open reading frame encoded the protein. As detd. by utilizing **fusion proteins** contg. truncated forms of FBP54, the primary Fn/Fgn-binding domain appears to be contained in residues 1 to 89. These data suggest that FBP54 may be a surface protein of streptococci that reacts with both Fn and Fgn and therefore may participate in the adhesion of **group A streptococci** to host cells.

L25 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:647482 CAPLUS
DOCUMENT NUMBER: 119:247482
TITLE: Protective immunogenicity of a recombinant hybrid protein containing a fragment of type-24 streptococcal M protein and the B subunit of Escherichia coli labile toxin
AUTHOR(S): **Dale, James B.**; Chiang, Elbert C.; Lederer, James W.; Bronze, Michael S.
CORPORATE SOURCE: Veterans Affairs Med. Cent., Univ. Tennessee, Memphis, TN, 38104, USA
SOURCE: Vaccines 93, [Annu. Meet.], 10th (1993), Meeting Date 1992, 409-12. Editor(s): Ginsberg, Harold S. Cold Spring Harbor Lab.: Cold Spring Harbor, N. Y.
CODEN: 59HUAJ
DOCUMENT TYPE: Conference
LANGUAGE: English
AB A **fusion** gene was constructed that encodes the entire B subunit of E. coli heat-labile toxin and 12 N-terminal amino acids of type-24 **streptococcal M protein.** The purified hybrid protein reacted with antibodies against both LT-B and pep **M24**. Rabbits immunized with LT-B-**M24** developed high titers of protective antibodies against type-24 streptococci.

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